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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,430,910**, on June 3, 2003, by **INFECTIO DIAGNOSTIC (IDI) INC.**, assignee of Mario Leclerc, Hoang-Anh Ho and Maurice Boissinot, for "Optical Sensors Based on Hybrid Aptamer/Conjugated Polymer Complexes ".

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ABSTRACT OF THE DISCLOSURE

A new selective, rapid and highly sensitive method (as few as 2×10^{-15} mole) for the detection of human α-thrombin is disclosed, based on the formation of hybrid anionic aptamer/cationic polythiophene complexes. Advantageously, this new method does not require any chemical reaction on the probes or the analytes and is based on conformational modifications of the conjugated backbone of a cationic poly(3-alkoxy-4-methylthiophene), when mixed with ss-DNA and a target protein.

TITLE OF THE INVENTION

OPTICAL SENSORS BASED ON HYBRID APTAMER/CONJUGATED POLYMER COMPLEXES

FIELD OF THE INVENTION

[0001] The present invention relates to optical sensors. More specifically, the present invention is concerned with optical sensors based on hybrid aptamer/conjugated polymer complexes.

BACKGROUND OF THE INVENTION

[0002] Intense research is being carried out worldwide with the goal of developing rapid, simple, specific, and sensitive detection tools for medical diagnostics and biomedical research applications. Fundamentally, most analytical tests and immunoassays rely on molecular recognition and its transduction into a measurable output. Among all the possible molecular recognition elements, artificial nucleic acid ligands (aptamers) have recently attracted a lot of interest due to their capability of binding various metal ions, amino acids, drugs, proteins, as well as other molecules having high affinity and specificity.[1-11]

[0003] Aptamers are usually isolated from combinatorial libraries of synthetic nucleic acids by an iterative process of adsorption, recovery, and amplification coined as SELEX (*Systematic Evolution of Ligands by Exponential Procedure*). Aptamer-based ligands constitute highly promising candidates for the specific detection of various molecules. Additionally, they can also be used in competition binding assays, such as for example in high-throughput screening assays[7], for the identification of new potential drugs capable of displacing the aptamers from their targets.

[0004] The above-mentioned approaches, however, require adequate transducing (*i.e.* reporting) elements in order to generate a physically measurable signal resulting from the recognition event. Binding of an aptamer to a target protein, for example, has been detected by using fluorescence (*e.g.* molecular beacons[12-13]) or by using a quartz microbalance.[14] In most cases, however, these methods either involve a tagging process or sophisticated experimental techniques. Furthermore, it is worth noting that labeling with various functional groups may even compromise the binding properties of the aptamers.

[0005] There thus remains a need to develop a rapid, simple, specific and sensitive detection tool capable of transducing the binding of an aptamer to its target into a clear signal.

[0006] The present invention seeks to meet these and other needs.

[0007] The present invention refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0008] The present invention relates to optical sensors based on hybrid aptamer/conjugated polymer complexes. More specifically, the present invention relates to the use of a water-soluble cationic polythiophene as a "polymeric stain" capable of specifically transducing the binding of an aptamer to its target into a clear optical (colorimetric or fluorometric) signal.

[0009] Further scope and applicability will become apparent from the detailed description given hereinafter. It should be understood however, that

this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

[0011] Figure 1A shows photographs of polymer **1**: (a) alone; (b) in the presence of X1 in LiCl 0.01M; (c) in the presence of X1 in NaCl 0.01M; (d) in the presence of X1 in KCl 0.01M; and (e) in the presence of X1 in RbCl 0.01M. Figure 1B shows the UV-Vis absorption spectra of polymer **1** (2.9×10^{-9} mole on a monomer unit basis) in the presence of X1 (1.9×10^{-10} mole of the 15-mer) and different salts in 100 µL of water at 25 °C;

[0012] Figure 2 shows the complexation between unfolded anionic ss-DNA and polymer **1**, as well as the complexation between unfolded anionic ss-DNA and polymer **1** in the presence of potassium ions;

[0013] Figure 3 illustrates the specific detection of human α-thrombin using ss-DNA thrombin aptamer X1 and positively-charged polymer **1**;

[0014] Figure 4 illustrates: (a) the UV-Vis absorption spectrum of polymer **1** in water at 25 °C; (b) the UV-Vis absorption spectrum of a complex (1/1/1) human thrombin/X1/polymer **1**, in water at 25 °C (c) the UV-Vis absorption spectrum of a mixture of human thrombin/X2/polymer **1**, in water at

25 °C; (d) the UV-Vis absorption spectrum of a mixture of BSA/X1/ polymer 1; of 1, in water at 25 °C; and

[0015] Figure 5 illustrates the fluorescence spectrum measured at 5 °C of (a) polymer 1; (b) human thrombin/X1/polymer 1 complex; (c) human thrombin/X2/polymer 1 mixture; (d) X1/polymer 1 complex in water.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

[0016] Single-stranded DNA (aptamer) can specifically bind potassium ions or human α-thrombin. When binding takes place, the aptamer undergoes a conformational transition from an unfolded to a folded structure. This conformational change of the negatively-charged oligonucleotide can be detected by adding a water-soluble, cationic polythiophene derivative which transduces the new complex formation into an optical (colorimetric or fluorometric) signal without any labeling of the probe or of the target.

[0017] The present invention relates to optical sensors based on hybrid aptamer/conjugated polymer complexes. More specifically, the present invention relates to the use of a water-soluble cationic polythiophene as a "polymeric stain" capable of specifically transducing the binding of an aptamer to its target into a clear optical (colorimetric or fluorometric) signal. The optical sensors do not require any chemical reaction to take place on the probes or with the analytes. Instead it is based on different conformational structures and different electrostatic interactions between a cationic poly(3-alkoxy-4-methylthiophene) derivative and anionic single-stranded oligonucleotides.

[0018] The use of the optical sensors of the present invention, for instance, allows to specifically detect as few as 2×10^{-15} moles of human

thrombin in only a few minutes and could be easily adapted for use in many other chemical or biochemical targets.

[0019] The cationic, water-soluble, electroactive, and photoactive polymer **1** (see Figure 1) was prepared according to known literature procedures.[15] As is observed for most poly(3-alkoxy-4-methylthiophene)s,[16-19] polymer **1** exhibits chromic properties (color changes) which are due to conformational changes of the flexible conjugated backbone. Moreover, polymer **1** is known to display important optical changes when complexed to ss-DNA or ds-DNA[15], making it a promising candidate for transducing the binding of an aptamer to a given target.

[0020] The monovalent potassium cation is known for its folding-inducing properties in several classes of nucleic acids.[20,21] As shown in Figure 1, an aqueous solution of polymer **1** is yellow with a maximum absorption (λ_{max}) at 402 nm (Figure 1A,a and B,a). This absorption maximum at a relatively short wavelength is related to a random-coil conformation of the polythiophene derivative, as any twisting of the conjugated backbone leads to a decrease of the effective conjugation length.[16] A red color (λ_{max}= 527 nm) was observed in the presence of LiCl (Figure 1A,b and B,b); NaCl (Figure 1A,c and B,c) or RbCl (Figure 1A,e and B,e) and ss-DNA (X1: 5'-GGTTGGTGTGGTTGG-3'). This red color shift is associated with a stoechiometric complexation between the unfolded anionic ss-DNA and the cationic polythiophene derivative (Figure 2, path A). Such stoechiometric polyelectrolyte complexes tend to be insoluble in the medium in which they are formed.[15] These red-violet aggregates (probably formed from planar polymer chains) possess an absorption spectrum similar to that obtained in the solid state. The optical properties (Figure 1A,d and B,d), however, are different when potassium ions are present. As a result of the formation of a folded structure (quadruplex form) of oligonucleotide X1,

stabilized by potassium ions (K^+), polymer **1** is able to wrap itself around this structure through electrostatic interactions (Figure 2, Path B). Similar results were also observed when the chloride counter-ion was replaced by a bromide or iodide counter-anion, indicative of the specificity of the detection towards potassium cations.

[0021] Human α-thrombin was selected as a further example since ss-DNA X1 (5'-GGTTGGTGTGGTTGG-3') is also known to be a specific binding sequence (*i.e.* an aptamer) of this protein, whereas another oligonucleotide ss-DNA (X2: 5'-GGTGGTGGTTGTGGT-3') is known to be a non-binding sequence.[22] A conformational change occurs when the aptamer binds to the thrombin molecule. Both NMR and X-ray diffraction studies have revealed that the aptamer adopts a compact unimolecular quadruplex structure with two G-quartets.[23,24] As shown in Figure 3, the specific detection of human α-thrombin is realized due to the formation of a quadruplex structure of the thrombin aptamer (X1). Accordingly, the 1:1:1 complex between polymer **1**, X1, and α-thrombin has a similar orange color and UV-Vis absorption spectrum than that induced by K^+ (Figure 4b). Human α-thrombin promotes the formation of a folded structure (quadruplex form) of thrombin aptamer X1, enabling cationic polymer **1** to wrap itself around this quadruplex structure, which seems to partially hinder the aggregation and planarization of the polymer **1** when in the presence of ss-DNA X1 (Figure 3, Path A). It is worth noting that only the stoechiometry of the aptamer (in terms of negative charges) and of polymer **1** (in terms of positive charges) has to be balanced, whereas an excess of α-thrombin does not influence its detection.

[0022] The specificity of the detection was verified by two control experiments carried out under identical conditions. In a first control experiment a non-binding sequence ss-DNA (X2: 5'-GGTGGTGGTTGTGGT-3') was used

(Figure 4,c) and in a second control experiment BSA (bovine serum albumin) was used (Figure 4,d). In both cases, an important red-shift toward lower energy (λ_{max}=505 nm) was observed. Furthermore, the color of these solutions was red-violet, which is typical of the planar and highly conjugated structure of the polythiophene backbone when mixed with unfolded ss-DNA (Figure 3, Path B and Figure 2, Path A). The detection limit of this colorimetric method is about $1x10^{-11}$ mole of thrombin in a total volume of *ca.* 100 µL (a concentration of about $1x10^{-7}$ M).

[0023] The fluorescent properties of conjugated polymers can be utilized to detect very small quantities of analytes.[25-29] The fluorometric detection of the binding of the thrombin aptamer to human α-thrombin is possible because the fluorescence of poly(3-alkoxy-4-methylthiophene) is quenched in the planar, aggregated form.[15-19] The yellow, random-coil form of polymer **1** is fluorescent (Figure 5,a) with an emission maximum at 525 nm. When non-specific thrombin aptamer (X2) is used (Figure 5,c), or in the absence of human thrombin (Figure 5,d), the red-violet, highly conjugated form has a much lower fluorescence intensity and the maximum of emission is red-shifted (λ_{em}=590 nm). However, when the 1:1:1 complex (human thrombin/thrombin aptamer X1/polymer **1**) is formed (Figure 5,b), the resulting orange intermediate form is less fluorescent than the yellow form but more fluorescent (*ca.* a six-fold increase) than the red-violet form.This higher intensity of emission could be related to a partially planar conformation of the polythiophene chain, but with less aggregation of the chains.[30]

[0024] The use of a standard spectrofluorimeter provides for a detection limit of 2×10^{-15} mole (a concentration of $1x10^{-11}$ M in 200 µL) of human α-thrombin.

Experimental section

UV-Vis measurements

[0025] All UV-Vis absorption spectra were taken using a Hewlett-Packard (model 8452A) spectrophotometer.

Detection of cations

[0026] In a quartz quartz cell having an optical path length of 1.0 cm, 4μL [2.9×10^{-9} mole (based on negative charges)] of 15-mer X1 were added to 100μL of an aqueous solution of a given alkali metal cation (10 mM) (chloride salts), followed by the addition of 4μL [2.9×10^{-9} mol (based on positive charge)] of a solution of cationic polymer **1**. All UV-Vis absorption spectra were recorded at room temperature.

Detection of human α-thrombin

[0027] In a UV quartz cell having an optical path length of 1.0 cm, 1.9×10^{-10} mole of human α-thrombin (Haematologic Technologies Inc.) (the initial concentrated solution was diluted with sterilized water to obtain the appropriate concentration) and 2.9×10^{-9} mole (based on negative charges or 1.9×10^{-10} mole of 15-mer) of ss-DNA thrombin aptamer X1 were mixed in 100 μL of pure water at 25°C. This was followed by the addition of 2.9×10^{-9} mole (based on charge repeat unit) of polymer **1**, to form a complex (1/1/1).

[0028] Two control experiments were carried out using a non-specific sequence X2 and BSA (bovine serum albumin, obtained from Sigma), under identical conditions.

Fluorescence measurements

[0029] All fluorescence spectra were recorded on a Carry Eclipse (Varian Inc.) spectrofluorimeter. The excitation was performed at 420 nm.

[0030] In fluorescence cell having an optical path length of 3.0 mm, 3.8×10^{-10} mole of human thrombin and 5.7×10^{-9} mole (based on monomeric negative charge or 3.8×10^{-10} mol of 15-mer) of ss-DNA thrombin aptamer X1 were mixed in 200 µl of pure water, followed by the addition of 5.7×10^{-9} mole (based on charge repeat unit) of polymer 1. The fluorescence spectrum of all mixtures was recorded at 5 °C. For the lower concentration of human α-thrombin, the excitation was performed at 420 nm, and the fluorescence emission intensity was measured at 584 nm (without recording the entire emission spectrum).

[0031] Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

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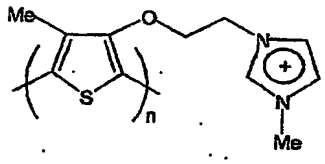
WHAT IS CLAIMED IS:

1. A method for detecting human α-thrombin comprising the steps of:

a) contacting said human α-thrombin with a complementary target to form a quadruplex structure;

b) contacting said quadruplex structure with a cationic polymer having the following formula:



wherein "n" is an integer ranging from 3 to 100; and

c) detecting a change in UV-Vis or fluorescence as an indication of the presence of said human α-thrombin.

2. An optical sensor for detecting a protein comprising a single stranded aptamer and a water-soluble, cationic polythiophene derivative, said aptamer being complementary to said protein.

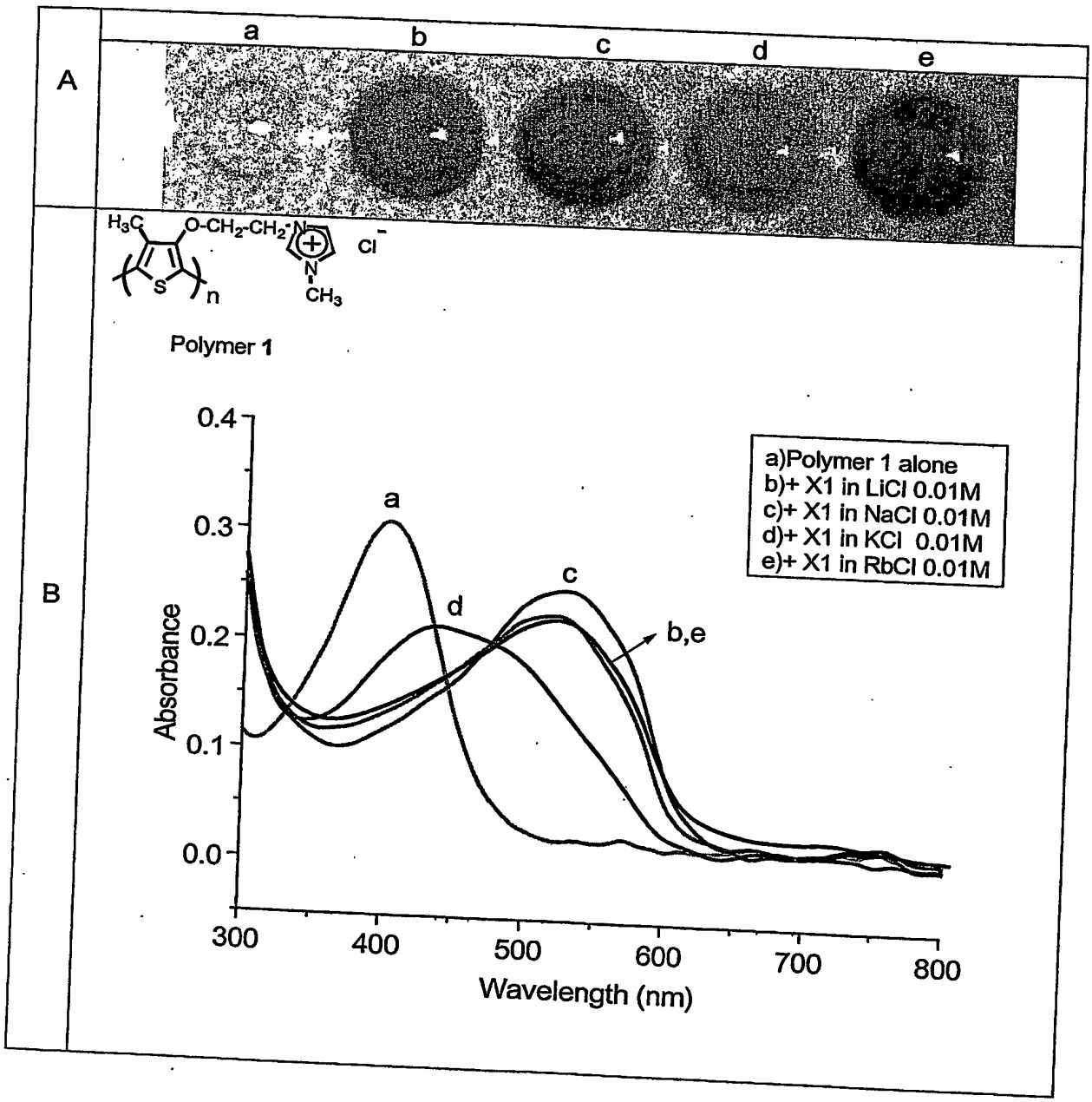

A
a
b
c
d
e
B
Polymer 1
a)Polymer 1 alone
b)+ X1 in LiCl 0.01M
c)+ X1 in NaCl 0.01M
d)+ X1 in KCl 0.01M
e)+ X1 in RbCl 0.01M
Absorbance
Wavelength (nm)
0.0
0.1
0.2
0.3
0.4
300
400
500
600
700
800
b,e

Figure 1

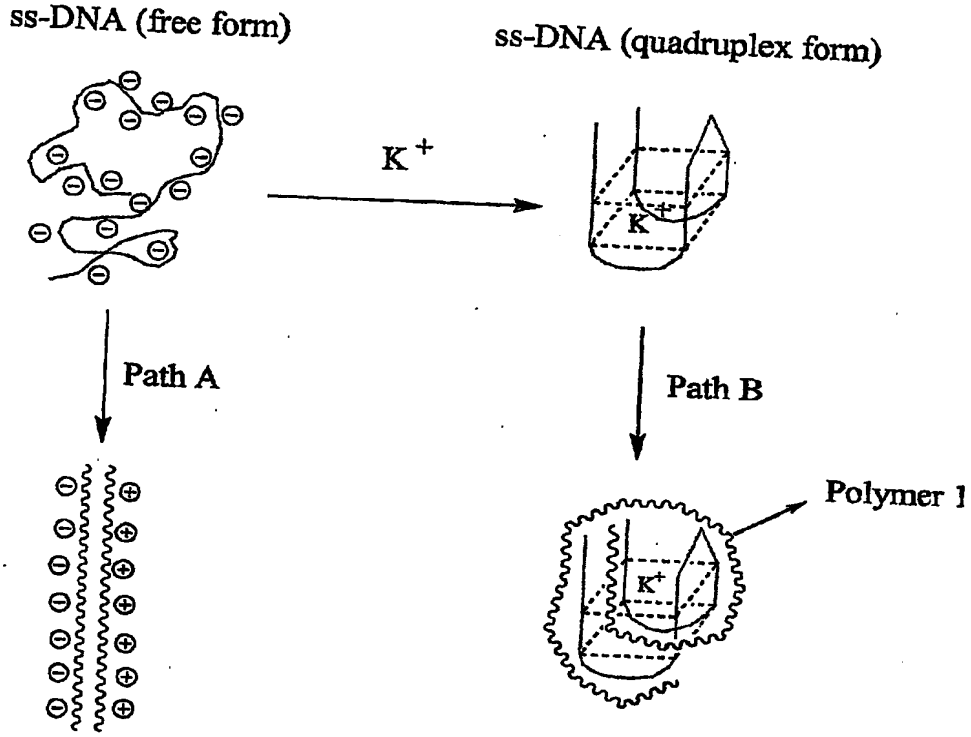

ss-DNA (free form)
ss-DNA (quadruplex form)
K+
K+
Path A
Path B
Polymer 1
K+

Figure 2

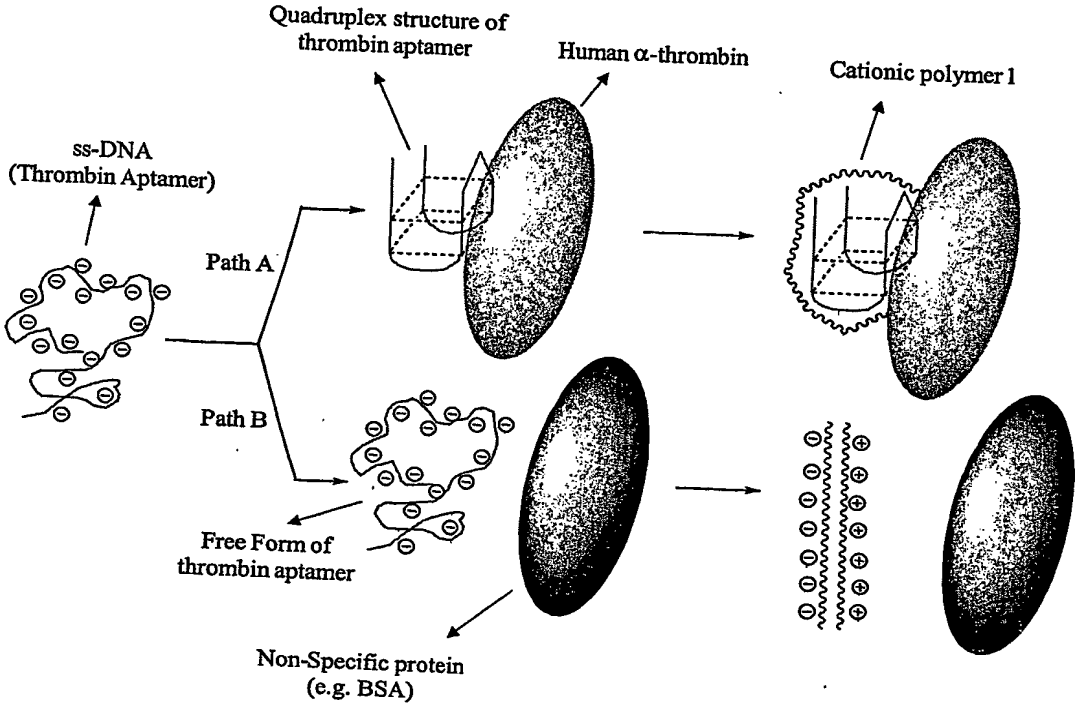

Quadruplex structure of thrombin aptamer
Human α-thrombin
Cationic polymer 1
ss-DNA (Thrombin Aptamer)
Path A
Path B
Free Form of thrombin aptamer
Non-Specific protein (e.g. BSA)

Figure 3

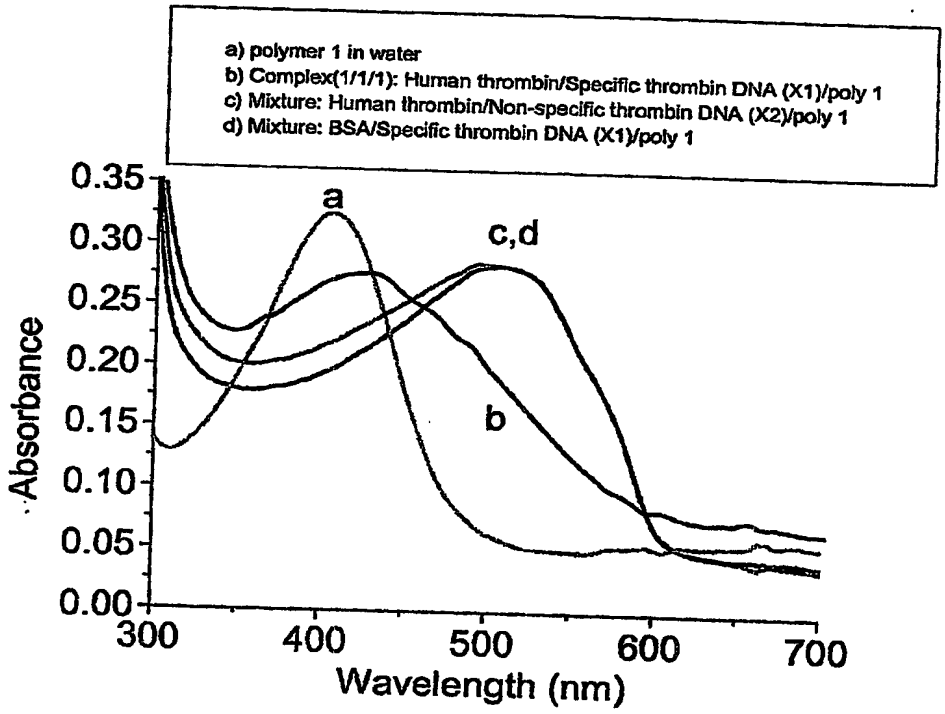

a) polymer 1 in water
b) Complex(1/1/1): Human thrombin/Specific thrombin DNA (X1)/poly 1
c) Mixture: Human thrombin/Non-specific thrombin DNA (X2)/poly 1
d) Mixture: BSA/Specific thrombin DNA (X1)/poly 1
Absorbance
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
300
400
500
600
700
Wavelength (nm)
a
c,d
b

Figure 4

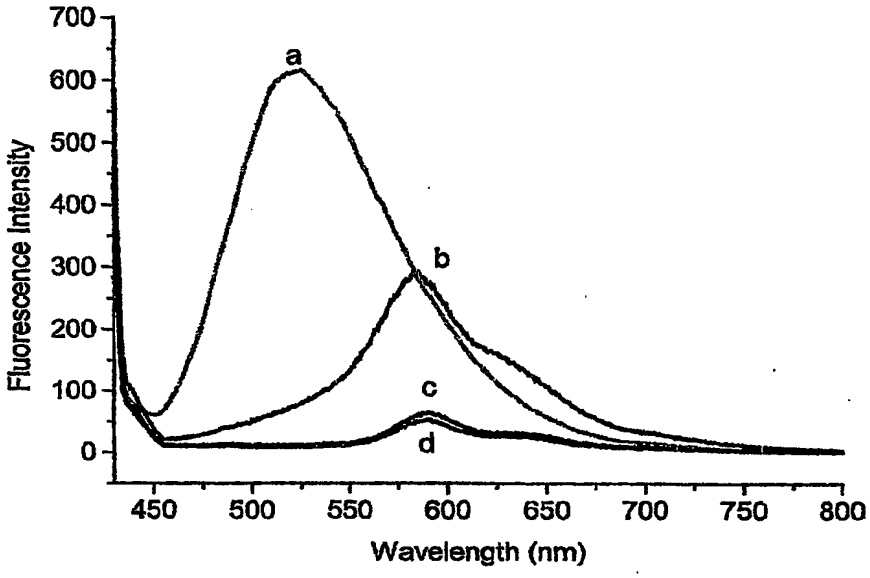

700
600
500
400
300
200
100
0
Fluorescence Intensity
a
b
c
d
450
500
550
600
650
700
750
800
Wavelength (nm)

Figure 5